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Long-Alkyl-Chain Quaternary Ammonium Lactate Based Ionic Liquids

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Abstract: A new group of quaternary ammonium lactate based ionic liquids have been prepared and characterized. Didecyldimethylammonium (DDA) and benzalkonium (BA) D,L- and L-lactates are air-stable, hydrophilic, surface-active salts. They are very effective antibacterial and antifungal agents, especially the DDA lactates, against *Streptococcus mutants* and *Candida albicans*. Their activities are comparable or more effective than the original benzalkonium chloride. In addition, they have been shown to be good insect-feeding deterrents. However, they are poor antifungal agents for wood preservation. The toxicity of the DDA and BA lactates has also been studied and the results are presented in this paper.

Keywords: biological activity · green chemistry · ionic liquids · lactates · quarternary ammonium salts

Introduction

Ionic liquids (ILs) have received much attention in recent years owing to their unique chemical and physical properties, such as low vapor pressure, high chemical and thermal stability, low melting points with a large liquid range, and their ability to dissolve a wide range of organic and inorganic compounds.[1–9] Typically, ILs consist of large, highly asymmetric organic cations and bulky anions. The solubilization ability, hydrophobicity, and hygroscopicity can be fine-tuned by simply modifying the cation and anion that form the resulting ILs. The IL field is dominated by imidazolium, quaternary ammonium, pyridinium, and phosphonium salts.

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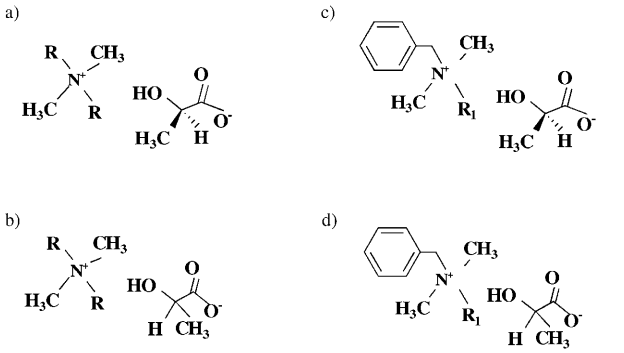
Owing to the strong interest in the application of ionic liquids, new, cheaper, multifunctional, and environmentally friendly ILs are preferred. Quaternary ammonium-based ionic liquids seem to be an answer to this demand. Quaternary ammonium salts (quats) are well known and widely used as cationic surfactants, antiseptics, sanitizers, softeners, phase-transfer catalysts, and conditioning agents in hair cosmetics. Recently, new applications for quats have been found in ILs.[10–13] We propose a novel organic class of quat-based ILs derived from common quaternary ammonium cations with GRAS (generally recognized as safe) materials (taken from the pharmaceutical and food additive industries), that is, lactate anions.

Lactic acid (2-hydroxypropanoic acid), also known as milk acid, plays a role in several biochemical processes. It is chiral and occurs as two optical isomers D (−) and L (+). The L (+) form plays an important part in carbohydrate metabolism. Lactic acid as an α-hydroxy acid (AHA) can lose a proton from the acidic group in solution to produce the lactate anion. Commercially used lactic acid is derived by using bacteria, such as *Bacillus acidilacti*, *Lactobacillus delbueckii*, or *Lactobacillus bulgaricus* to ferment carbohydrates, such as glucose, sucrose, and lactose. The lactic acid used in commerce is usually an optically inactive racemic mixture of the two isomers. It is produced commercially for use in pharmaceuticals and foods, in leather tanning and textile dyeing, and in making plastics, solvents, inks, and lacquers. Lactic acid is cheap, commercially available, nontoxic, and pharmaceutically acceptable (LD_{50}: rat oral 3543 mg kg^{-1}; mouse oral 4875 mg kg^{-1}[14]).

In this paper we present novel long-alkyl-chain quaternary ammonium lactate based ionic liquids along with their properties and potential applications.

Results and Discussion

Synthesis and characterization: Lactates with didecyldimethylammonium (DDA) and benzalkonium (BA) cations (Scheme 1) were synthesized by anion-exchange reaction (at



Scheme 1. Structures of the prepared lactates: a) [DDA][L-lactate], b) [DDA][D,L-lactate], c) [BA][L-lactate], and d) [BA][D,L-lactate] ($R = C_{10}H_{21}$, $R^1 = C_{12}H_{25}$ (60%), $R^1 = C_{14}H_{29}$ (40%)).

room temperature in water) of the commercially inexpensive and widely used quaternary chloride salts, [DDA][Cl] and [BA][Cl], with lactic acid and potassium hydroxide with an efficiency of over 90%. Potassium chloride as a byproduct was removed from an anhydrous acetone solution with success. [DDA][D,L-lactate], [DDA][L-lactate], [BA][D,L-lactate], and [BA][L-lactate] are soluble in water and are reported here for the first time.

The products were dried in vacuum at 80°C for 24 h and stored over P_4O_{10}. The water content, determined by Karl-Fischer measurements, was found to be less than 500 ppm. The prepared salts were characterized by ^1H and ^{13}C NMR spectroscopy and elemental analysis. The physicochemical properties of the prepared lactates are presented in Table 1.

Table 1. Physicochemical properties of the prepared lactates.[a]

	T_g [°C]	T_{onset} [°C]	T_{endset} [°C]	ρ[b] [g mL^{-1}]	η[c] [cP]	K[b] [μS cm^{-1}]
[DDA][L-lactate]	−56	208	256	0.944	2900	12.7
[DDA][D,L-lactate]	−56	200	278	0.944	1600	12.7
[BA][L-lactate]	−36	181	218	0.999	9400	6.8
[BA][D,L-lactate]	−36	181	223	0.999	11200	6.8

[a] The glass transition temperature (T_g) was determined by DSC on heating. The decomposition temperature (T_{onset}) was determined from the onset of decomposition; T_{endset} is the temperature of decomposition. ρ is the density, η the viscosity, and K is the conductivity. [b] Measured at 20°C. [c] Measured at 25°C.

Differences in data occur between the DDA and BA cations. On the other hand, parameters obtained for the D,L- and L-lactate anions are comparable. The products are liquids at room temperature, have high viscosities, and glass-forming temperatures below zero. The existence of an organic anion in ILs does not reduce the thermal stability. The densities of the prepared hydrophilic ILs are almost equal to the density of water and their conductivities are not very high. The synthesized ILs are air- and moisture-stable salts. However, they can decompose to the cation and anion on contact with strong non-organic acids, such as HCl and HNO_3, at raised temperatures.

Surface activity: The surface activity parameters of the DDA and BA lactates in aqueous solution are presented in Table 2. Representative plots of surface tension as a function of log C (C is the concentration of surfactant, mol L^{-1}) are shown in Figure 1. The surface excess concentrations (Γ_{max}) of the DDA and BA lactates in aqueous solution were calculated from the slope of the linear portion of the γ versus log C plots by using the Gibbs isotherm [Eq. (1), in which Γ_{max} is the surface excess concentration at the saturat-

Table 2. The cmc, surface tension (γ_{cmc}), surface excess concentration (Γ_{max}), and area per molecule (A_{min}) of the prepared lactates in aqueous solution at 25°C.

	cmc [10^{-3} mol L^{-1}]	γ_{cmc} [mN m^{-1}]	Γ_{max} [10^{-6} mol m^{-2}]	A_{min} [10^{-19} m^2]
[DDA][L-lactate]	1.00	28.3	3.59	4.63
[DDA][D,L-lactate]	0.79	27.0	3.13	5.31
[BA][L-lactate]	5.01	38.5	3.64	4.57
[BA][D,L-lactate]	3.98	37.4	2.48	6.70

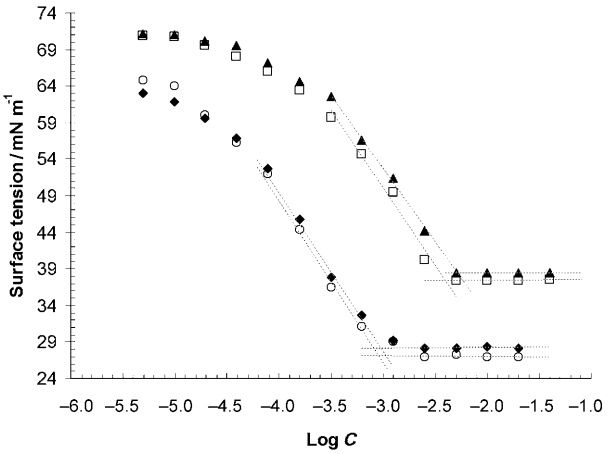


Figure 1. Surface tension as a function of concentration for the quaternary ammonium lactates in aqueous solutions at 25°C: [DDA][L-lactate] (◆), [DDA][D,L-lactate] (○), [BA][L-lactate] (▲), and [BA][D,L-lactate] (□). The straight lines are drawn to determine the cmc values.

ed interface, R is the gas constant, and T the absolute temperature]. From Γ, the minimum surface occupied by a molecule at the interface A_{min} can be calculated by using Equation (2) in which N_A is Avogadro's number.

$$\Gamma_{max} = -\frac{1}{RT}\frac{d\gamma}{d(\ln c)} \quad (1)$$

$$A_{min} = \frac{1}{\Gamma_{max} N_A}$$

The critical micelle concentrations (cmc), the corresponding surface tensions (γ_{cmc}), the surface excess concentrations (Γ_{max}), and the occupied surface areas per molecule (A_{min}) are summarized in Table 2.

The cmc values obtained from the plots of [DDA][L-lactate] and [DDA][D,L-lactate] are not identical but are quite close. A similar situation is observed for solutions of [BA][L-lactate] and [BA][D,L-lactate]. In the case of solutions of [DDA][L-lactate] and [DDA][D,L-lactate], the surface tension decreases from the value for water to a minimum of 28.3 and 27.0 mN m^{-1}, respectively. After this point the surface tension reaches a plateau. For solutions of [BA][L-lactate] and [BA][D,L-lactate], higher values are obtained: 38.5 and 37.4 mN m^{-1}, respectively. This means that the DDA lactate exhibits larger intermolecular hydrophobic interactions, making it easier to form aggregates in water than [BA][lactate]. Concerning the γ_{cmc} values, they are dependent on the isomeric form of the lactate. For [DDA][lactate] and [BA][lactate], the γ_{cmc} values are lower for the racemic isomers, [DDA][D,L-lactate] and [BA][D,L-lactate]. The areas per molecule A for both racemic forms is higher than the values of the corresponding [L-lactate] form, which indicates that molecules of [DDA][L-lactate] and [BA][L-lactate] are more tightly packed at the water/air interface. [DDA][lactate] has lower cmc and γ_{cmc} values than [BA][lactate], which indicates it has better surface properties. However, both cmc and γ_{cmc} values of aqueous solutions of the DDA and BA lactates are of the same order as the cmcs of cationic surfactants. For instance, the cmc of hexadecyltrimethylammonium bromide (CTAB) is 0.9 mmol L^{-1}.[15] These results indicate that the DDA and BA lactates self-assemble easily in aqueous solutions. The cmcs obtained are similar or lower than values reported for ionic liquids in aqueous solutions in other papers.[16–19]

Table 3. MIC and MBC values[a] for DDA and BA lactates and for [BA][Cl].[b]

Strain		[DDA][L-lactate]	[DDA][D,L-lactate]	[BA][L-lactate]	[BA][D,L-lactate]	[BA][Cl][b]
cocci						
M. luteus	MIC	0.5	2	1	2	0.5
	MBC	2	2	8	16	4
S. aureus	MIC	0.2	0.5	1	2	1
	MBC	2	0.5	4	2	8
S. epidermidis	MIC	<0.1	0.2	1	1	0.5
	MBC	0.2	0.2	4	8	2
S. mutans	MIC	<0.1	<0.1	0.1	0.1	–
	MBC	1	<0.1	1	2	–
E. faecium	MIC	0.5	<0.1	0.5	2	2
	MBC	2	1	4	2	8
M. *catarrhalis*	MIC	1	1	0.5	2	0.2
	MBC	2	1	4	2	0.5
rods						
E. coli	MIC	0.2	<0.1	0.5	8	1
	MBC	0.5	<0.1	8	125	1
S. marcescens	MIC	16	16	62	125	62
	MBC	16	31	62	125	62
P. vulgaris	MIC	8	8	31	250	31
	MBC	16	8	31	250	31
P. aeruginosa	MIC	16	16	62	125	62
	MBC	31	16	62	125	62
B. subtilis	MIC	<0.1	<0.1	1	1	1
	MBC	<0.1	<0.1	1	1	1
fungi						
C. albicans	MIC	1	2	2	4	4
	MBC	2	2	8	62	31
R. rubra	MIC	1	1	2	2	8
	MBC	2	2	4	62	31

[a] In mg L^{-1}, the number of microorganisms in 1 mL ranged from 10^4 to 10^5. [b] Benzalkonium chloride.

Biological activity: The antimicrobial activity was estimated for the DDA and BA lactates. The minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC) were established. The studies were conducted on eleven strains of bacteria and two strains of fungi. The MIC and MBC values of the prepared salts and the commercially available [BA][Cl] are compared in Table 3. The results demonstrate that the DDA and BA lactates are very effective agents against bacteria and fungi. Their activities are comparable or more effective than the original chloride [BA][Cl]. The data show that the most useful is DDA lactate, regardless of the anion's form: D,L-lactate or L-lactate. The very low values obtained for MIC and MBC show the high potential of our products in disinfection.

Wood preservation: Exceptional results obtained from microbiological research stimulated us to test the prepared lactates for wood preservation. Fungal growth by the agar-plate method was first determined for the fungal strains *S. pityophila*, *T. versicolor*, and *C. puteana.* The toxicity data (ED_{50}, ED_{100}, and LD) of the synthesized lactates and sodium lactate are presented in Table 4. The results show that the lactates are poor antifungal agents for wood preservation. In this case the lactate anion increased the activity of the cation. Nowadays, chlorides and bromides of DDA and BA are used for wood preservation with success. The latest research showed that nitrates are also effective.[12]

Table 4. The antifungal effective doses (ED_{50} and ED_{100} in ppm) and the lethal dose (LD in ppm) for *S. pityophila*, *T. versicolor*, and *C. puteana*.

Lactate	*Coniophora puteana*			*Trametes versicolor*			*Sclerophoma pityophila*		
	ED_{50}	ED_{100}	LD	ED_{50}	ED_{100}	LD	ED_{50}	ED_{100}	LD
[DDA][L-lactate]	50	>5000	>5000	100	>5000	>5000	25	500	2500
[DDA][D,L-lactate]	50	5000	>5000	100	>5000	>5000	10	500	2500
[BA][L-lactate]	250	5000	>5000	100	2500	2500	25	250	2500
[BA][D,L-lactate]	250	>5000	>5000	250	2500	2500	25	250	2500
sodium lactate	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000

Insect-feeding deterrent activities: The deterrent activities of the DDA and BA lactates towards *Tribolium confusum* (larvae and adults), *Sitophilus granarius* (adults), and *Trogoderma granarium* (larvae) are presented on the basis of the amount of food consumed. In all variants, three deterrent coefficients were calculated as follows:

1) the absolute coefficient of deterrency, calculated from the no-choice test: $A=(CC-TT)/(CC+TT)\times 100$
2) the relative coefficient of deterrency, calculated from the choice test: $R=(C-T)/(C+T)$
3) the total coefficient of deterrency: $T=A+R$

in which C and CC are the amounts of food consumed from the control discs, and T and TT are the amounts of food consumed that has been treated with the tested ILs.

The deterrent activities were estimated by the criteria listed in Table 5 and described previously.[20] In addition, Table 6 lists the relative, absolute, and total coefficients for the natural deterrent azadirachtin, treated as a standard. Azadirachtin (tetranortriterpenoid) was isolated from the seeds of the neem tree *Azadirachta indica* A. Juss., *Meliaceae*, and the chinaberry tree *Melia azadirachta* L. The antifeedant activity of this natural product has been described previously by Gill and Lewis.[21]

Table 5. Criteria for the estimation of deterrent activity based on the total coefficient.[20]

Total coefficient	Deterrent activity
200–151	very good
150–101	good
100–51	medium
50–0	weak

Table 6. Feeding deterrent activities of didecyldimethylammonium and benzalkonium lactates against *Tribolium confusum* (beetles, larvae), *Sitophilus granarius* (beetles), and *Trogoderma granarium* (larvae).

Lactate	Relative coefficient	Absolute coefficient	Total coefficient	Deterrent activity
Tribolium confusum (beetles)				
[DDA][L-lactate]	95.9	63.3	159.3	very good
[DDA][D,L-lactate]	96.1	76.5	172.6	very good
[BA][L-lactate]	96.6	1.8	98.4	medium
[BA][D,L-lactate]	96.1	6.4	102.5	good
azadirachtin[a]	100.0	85.0	185.0	very good
Tribolium confusum (larvae)				
[DDA][L-lactate]	79.6	81.5	161.1	very good
[DDA][D,L-lactate]	94.2	76.8	171.0	very good
[BA][L-lactate]	77.0	54.5	131.5	good
[BA][D,L-lactate]	94.6	40.1	134.7	good
azadirachtin[a]	100.0	88.4	188.4	very good
Sitophilus granarius (beetles)				
[DDA][L-lactate]	97.8	69.8	167.5	very good
[DDA][D,L-lactate]	98.2	72.9	171.0	very good
[BA][L-lactate]	97.6	15.1	112.7	good
[BA][D,L-lactate]	97.6	8.2	105.8	good
azadirachtin[a]	100.0	74.3	174.3	very good
Trogoderma granarium (larvae)				
[DDA][L-lactate]	94.9	92.4	187.3	very good
[DDA][D,L-lactate]	94.8	91.1	185.9	very good
[BA][L-lactate]	95.0	85.0	180.0	very good
[BA][D,L-lactate]	91.4	87.4	178.8	very good
azadirachtin[a]	100.0	94.2	194.2	very good

[a] Natural deterrent.

The deterrent activities of the DDA and BA lactates are dependent on the beetles or larvae tested. At the same time, the form of the cation is of importance. DDA lactates were active on a very good level in all cases, regardless of the form of the anions: D,L-lactate or L-lactate.

Toxicity: Acute oral toxicity studies of [DDA][L-lactate] and [BA][L-lactate] were performed on rats, in compliance with the OECD Guideline for Testing of Chemicals No 420 (Fixed Dose Method).[22] The results reported below show that [DDA][L-lactate] and [BA][L-lactate] can be included in Category 4, in line with the guidelines of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). GHS categories were integrated into the GHS Acute Toxicity Scheme from which appropriate elements relevant to transport, consumer, worker, and environment protection can be selected. Substances are assigned to one of the five toxicity categories on the basis of LD_{50} (oral, dermal) or LC_{50} (inhalation). Category 1, the most severe toxicity category, has cut-off values currently used primarily by the transport sector for classification for packing groups. Category 5 is for chemicals that are of relatively low acute toxicity but which, under certain circumstances, may cause a hazard to susceptible populations.[23]

Clinical signs: In preliminary experiments performed on rats, administration of [DDA][L-lactate] at a dose of 2000 mg kg^{-1} body weight (b.w.) was followed by no signs of toxicity and the female died in the course of the fourth hour. On the other hand, administration of the studied material at a dose of 300 mg kg^{-1} b.w. was followed in one female by signs of toxicity including a rounded back and diarrhea between the fifth and twelfth day of observation, ophthalmic exudate (deposition of porphyrins) and outflow from nostrils (deposition of porphyrins) between the fifth and seventh day of observation, and the animal was difficult

to catch and had erect fur between the seventh and fourteenth day of observation. The female survived 14 d of observation.

Administration of [DDA][L-lactate] at a dose of 300 mg kg^{-1} b.w. to a further four females in the experiment proper was followed by the following signs of toxicity in female no. 5 on the fourth day: inertia; clear motile inactivity (the animal easily tolerated being removed from the cage); and dermal lividity on ears, extremities, and tail. Female no. 5 died on the fourth day. No signs of toxicity were noted in the remaining females. They survived the 14 d of observation. Individual results related to weight gain in the animals are listed in Table 7.

Table 7. Tests for the acute oral toxicity of [DDA][L-lactate] in rats.

Dose [mg kg^{-1} b.w.]	Rat no.	Animal body weight [g] original	after 7 d	after 14 d	Difference[b]
2000	1[a]	179	–	–	–
300	1[a]	172	153	147	−25
	2	180	190	207	27
	3	186	210	213	27
	4	191	209	221	30
	5	186	–	–	–

[a] Females in the preliminary experiment. [b] Difference in body weight between day 0 and day 14.

After administering in a preliminary experiment [BA][L-lactate] at a dose of 2000 mg kg^{-1} b.w. to a single female no signs of toxicity were recorded on the day of administration, but the female died after 24 h. In the same experiment a female administered with the material at a dose of 300 mg kg^{-1} b.w. manifested no clinical signs of toxicity on the day of administration or during the entire observation period. The female survived the 14 d of the experiment.

In the experiment proper administration of [BA][L-lactate] at a dose of 300 mg kg^{-1} b.w. to another four females was followed by signs of toxicity in all the females but only on the first day. They all survived the 14 d of the experiment. Individual results related to weight gain in the animals are listed in Table 8.

Table 8. Tests for the acute oral toxicity of [BA][L-lactate] in rats.

Dose [mg kg^{-1} b.w.]	Rat no.	Animal body weight [g] original	after 7 d	after 14 d	Difference[b]
2000	1[a]	179	–	–	–
300	1[a]	183	199	220	37
	2	175	194	203	28
	3	187	188	219	32
	4	180	199	210	30
	5	179	193	208	29

[a] Females in the preliminary experiment. [b] Difference in body weight between day 0 and day 14.

Pathology: Macroscopic studies on animals given [BA][L-lactate] documented no pathological alterations. Alterations, however, were noted following administration of [DDA][L-lactate]. In one female (no. 5), which died, a fibrin deposit was noted on the liver and spleen capsules and on the gastric peritoneum as well as peritoneal adhesions. Among the four females sacrificed following a 14 d period peritoneal adhesions (accretion of the stomach and liver), peritonitis, and intestinal hyperaemia were noted in one female only.

Conclusion

A novel group of quaternary ammonium lactate based ionic liquids have been synthesized and their biological activities investigated. Didecyldimethylammonium (DDA) and benzalkonium (BA) D,L- and L-lactates were found to be very effective antibacterial and antifungal agents. Their activities were comparable or more effective than the original chloride [BA][Cl], especially DDA lactates, against *Streptococcus mutants* and *Candida albicans*. Moreover, these salts were shown to be good insect-feeding deterrents. In addition, our results indicate that DDA and BA lactates self-assemble easily in aqueous solution. Their cmcs are similar or lower than the values of aqueous solutions of classic cationic surfactants and other ionic liquids. However, our results have shown that these lactates are poor antifungal agents for wood preservation. The acute oral toxicity studies performed with [DDA][L-lactate] and [BA][L-lactate] showed that these salts can be included in Category 4 of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Experimental Section

General: ^{1}H NMR spectra were recorded on a Mercury Gemini 300 spectrometer operating at 300 MHz with tetramethylsilane as the internal standard. ^{13}C NMR spectra were obtained with the same instrument at 75 MHz. CHN elemental analyses were performed at the A. Mickiewicz University, Poznan (Poland). The water content was determined by using an Aquastar volumetric Karl-Fischer titration with Composite 5 solution as the titrant and anhydrous methanol as the solvent. Glass transitions were measured by using a Perkin–Elmer differential scanning calorimeter. Samples were sealed in aluminum pans and scanned at a rate of 10 K min^{-1} in an argon atmosphere. Onset and endset temperatures of decomposition were established by using a Mettler Toledo TGA/SDTA 851e apparatus. The TGA data were collected at 10 K min^{-1} under argon. Conductivity measurements were based on impedance spectroscopy in which the complex resistivities were generated by using the a.c. current amplitude. The viscosity of each salt was measured by using a Brookfield Digital Viscosimeter, Model DV-II. Optical rotations were measured with a Perkin–Elmer 241 polarimeter.

General synthetic procedure: Lactic acid (0.1 mol of an 85% aq. solution) and potassium hydroxide (0.1 mol) in water (50 mL) were added to a 1.5 L reaction flask equipped with a reflux condenser, stirring bar, and thermometer. The mixture was heated at reflux until HPLC analysis showed no signal of the oligomeric lactic acid. Then a 50% solution of [DDA][Cl] or [BA][Cl] (0.1 mol) in water was added and the mixture was stirred at room temperature for 5 h. The water was removed under reduced pressure (70 °C, 30×10^{2} Pa). Anhydrous acetone was added and the mixture was allowed to stand overnight at room temperature. The crystalline potassium chloride was removed by filtration and the acetone by distillation.

Didecyldimethylammonium D,L-lactate ([DDA][D,L-lactate]): Yield: 90%, colorless product; ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.63$ (q, 1H), 3.29

(m, 4H), 3.04 (s, 6H), 1.63 (m, 4H), 1.26 (m, 28H), 1.11 (d, 3H), 0.88 ppm (t, 6H); ^{13}C NMR ([D$_6$]DMSO): δ=176.8, 62.7, 62.6, 49.9, 31.3, 28.9, 28.8, 28.7, 28.5, 25.8, 22.1, 21.7, 21.3, 13.9 ppm; elemental analysis calcd (%) for $C_{25}H_{53}NO_3$ (415.7): C 72.23, H 12.85, N 3.37; found: C 72.30, H 13.01, N 3.43.

Didecyldimethylammonium L-lactate ([DDA][L-lactate]): Yield: 94%, colorless product; $[\alpha]_D^{20}=-2.1°$ (c=1.0 in methanol); ^{1}H NMR ([D$_6$]DMSO): δ=3.63 (q, 1H), 3.32 (m, 4H), 3.05 (s, 6H), 1.64 (m, 4H), 1.26 (m, 28H), 1.11 (d, 3H), 0.88 ppm (t, 6H); ^{13}C NMR ([D$_6$]DMSO): δ=176.9, 62.8, 62.6, 49.9, 31.3, 29.0, 28.9, 28.8, 28.5, 25.8, 22.1, 21.7, 21.3, 13.9 ppm; elemental analysis calcd (%) for $C_{25}H_{53}NO_3$ (415.7): C 72.23, H 12.85, N 3.37; found: C 72.11, H 13.01, N 3.50.

Benzalkonium L-lactate ([BA][L-lactate]): Yield: 92%, colorless product; $[\alpha]_D^{20}=-2.8°$ (c=1.0 in methanol); ^{1}H NMR ([D$_6$]DMSO): δ=7.61 (m, 2H), 7.52 (m, 3H), 4.63 (s, 2H), 3.63 (q, 1H), 3.32 (m, 2H), 3.00 (s, 6H), 1.81 (m, 2H), 1.26 (m, 21H), 1.13 (d, 3H), 0.87 ppm (t, 6H); ^{13}C NMR ([D$_6$]DMSO): δ=177.1, 133.0, 130.1, 128.8, 128.4, 66.9, 66.0, 63.3, 49.0, 31.3, 29.11, 29.10, 29.06, 28.98, 28.86, 28.76, 28.57, 25.9, 22.1, 21.8, 21.4, 13.9 ppm.

Benzalkonium D,L-lactate ([BA][D,L-lactate]): Yield: 95%, colorless product; ^{1}H NMR ([D$_6$]DMSO): δ=7.53 (m, 5H), 4.60 (s, 2H), 3.66 (q, 1H), 3.32 (m, 2H), 2.98 (s, 6H), 1.78 (m, 2H), 1.25 (m, 21H), 1.13 (d, 3H), 0.89 ppm (t, 6H); ^{13}C NMR ([D$_6$]DMSO): δ=177.1, 133.0, 130.3, 128.4, 128.5, 66.9, 66.0, 63.3, 49.0, 31.3, 29.1, 29.08, 29.01, 28.9, 28.8, 28.6, 25.9, 22.1, 21.8, 21.4, 13.9 ppm.

Surface activity: Surface tension measurements were taken by using a Tracker drop tensiometer (I.T. Concept, Longessaigne, France, accuracy $\pm$0.01 mN m^{-1}) at 25°C. The surface tension was determined by using the shape drop method.[24] The principle of this method is to form an axisymmetric air bubble at the tip of a syringe needle. A computer drives the plunger position of the syringe using a motor drive into a thermostatted optical glass cuvette containing 7 mL of a solution in water. The image of the bubble (6 µL) is taken with a CCD camera and digitized. The surface tension (γ in mN m^{-1}) is calculated by analyzing the profile of the bubble according to the Laplace equation. The temperature was controlled by using a Fisherbrand FBH604 thermostatic bath (Fisher, Germany, accuracy $\pm$0.1°C). The values of the critical micelle concentration (cmc) and the surface tension at the cmc (γ_{cmc}) were determined from the intersection of the two straight lines drawn in the low- and high-concentration regions of the surface tension curves (γ versus log C curves) by using a linear regression analysis method.

Bioactivity tests: The following microorganisms were used: *Micrococcus luteus* NCTC 7743, *Staphylococcus aureus* NCTC 4163, *Staphylococcus epidermidis* ATCC 49134, *Streptococcus mutans* PCM 2502, *Enterococcus faecium* ATCC 49474, *Moraxella catarrhalis* ATCC 25238, *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 8100, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* NCTC 6749, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, and *Rhodothorula rubra* (Demml 1889, Lodder 1934). Standard strains were supplied by the National Collection of Type Cultures (NCTC), London, the American Type Culture Collection (ATCC), and the Polish Collection of Microorganisms (PCM). *Rhodothorula rubra* was obtained from the Department of Pharmaceutical Bacteriology, University of Medical Sciences, Poznan (Poland).

The antimicrobial activity was determined by the tube dilution method. A series of DDA and BA lactate dilutions were prepared in Mueller–Hinton broth (bacteria) or Sabouraud broth (fungi) media. Bacteria strains were cultured in Mueller–Hinton broth for 24 h and in Brain Heart Infusion broth for 48 h (*Streptococcus mutans*) and fungi in Sabouraud agar for 48 h. A suspension of the microorganisms at a concentration of 10^6 cfu mL was prepared from each culture and each dilution of the tested lactate was inoculated with one of the above-mentioned suspensions in a 1:1 ratio. Growth of the microorganisms (or the lack of growth) was determined visually after incubation for 24 h at 37°C (bacteria) or for 48 h at 28–30°C (fungi). The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC. Then, from each tube, one loopful was cultured on an agar medium with inactivates (0.3% lecithin, 3% polysorbate 80, and 0.1% cysteine L) and incubated for 48 h at 37°C (bacteria) or for 5 d at 28–30°C (fungi). The lowest concentration of lactate that does not support colony formation was defined as the MBC.

Wood preservation: The experiments were conducted with *Coniophora puteana* (Schum: Fr) Karst. strain BAM 15 (brown rot), *Trametes versicolor* (L.: Fr.) Pilát strain CTB 863 (white rot), and *Sclerophoma pityophila* (Corda) v. Höhn strain S231 (blue stain) obtained from the collection of the Wood Technology Institute, Poznan (Poland). The fungal growth rates were measured in 90 mm diameter dishes by using the agar-plate method.[25] A stock solution of each concentration was produced in sterile malt agar (1.5% agar and 4% malt extract), 20 mL of which was added to Petri dishes. For each strain, three replicate plates of each concentration of preservative were centrally inoculated with a 5 mm diameter disc taken from the submargin of 10-day-old malt agar plates. The tested concentrations of each compound in sterile malt agar were 10, 25, 50, 100, 250, 500, 750, 1000, 2500, and 5000 ppm. The plates were incubated at (22$\pm$1)°C in the dark. The duration of the test was determined by waiting for complete plate coverage, which took 10 d for *C. puteana*, 6 d for *T. versicolor*, and 12 d for *S. pityophila*. If growth had not begun on the preservative-containing agar after 10 or 12 d, the inoculum was removed and transferred to a fresh malt agar plate to determine the fungal viability. The effective doses, ED_{50} and ED_{100} (preservative concentrations retarding the fungal growth rate by 50 or 100%, respectively, in comparison to plates in which the toxicant had not been added), were calculated based on these results. The same method was employed to measure the lethal doses, LD (concentration causing death of the inoculum). Despite retarding the fungal growth rate by 100% (ED_{100}), the fungus may still remain alive. LDs were determined by reinoculation of a new nutrition substrate with the inoculum at the ED_{100} dose to establish the concentration that causes death of the inoculum.

Bioassays: The bioassay experiments were conducted with *Tribolium confusum* Duv. (larvae and adults), *Sitophilus granaries* L. *(adults)*, and *Trogoderma granarium* Ev. (larvae). They came from laboratory colonies reared in a chamber maintained at (26$\pm$1)°C and (60$\pm$5)% relative humidity on a wheat grain or whole-wheat meal diet.

Choice and no-choice tests for insect-feeding were conducted following a previously described procedure.[20] Wheat wafer discs (1 cm in diameter × 1 mm thick) were saturated by dipping either in ethanol only (control) or in a solution of the IL (1%) in ethanol to be tested. After evaporation of the solvent (30 min of air-drying) the wafers were weighed and offered to the insects in plastic boxes as the sole food source for 5 d. The feeding of insects was recorded under three sets of conditions: 1) On two control discs (*CC*), 2) on a choice between one treated disc (*T*) and one control disc (*C*; choice test), and 3) on two treated discs (*TT*; no-choice test). Each of the three experiments was repeated five times with 3 adults of *Sitophilus granarius,* 20 adults and 10 larvae of *Tribolium confusum,* and 10 larvae of *Trogoderma granarium.* The number of individual insects depended on the intensity of their food consumption. The adults used for the experiments were unsexed, 7–10 d old, and the larvae were 5–30 d old. After 5 d, the discs were reweighed and the average weight of eaten food was calculated.

Acute oral toxicity study: The Wistar rats (symbol lmp: WIST, stado outbred) used in these studies originated from a culture from the Medical Institute of Work in Lodz (Poland) and were kept in cages of the conventional type. Before the study, the animals were quarantined for a minimum 5 d and observed daily during this period. The animals were marked individually. During quarantine and the experiments, the animals were kept in a room conditioned with the following parameters: a temperature of 21–22°C, a relative air humidity of 40–75%, and artificial illumination consisting of 12 h light/12 h darkness. Rats were kept in cages with a plastic bottom and a wired superstructure with dimensions of 58 × 37 × 21 cm (length × width × height). The animals were kept in cages individually (in the observation study, with a dose of 2000 or 300 mg kg^{-1} b.w.) or with four rats per cage (in the main study, with a dose of 300 mg kg^{-1} b.w.). UV-sterilized wood shavings were used as litter. Each cage was equipped with a label containing information on the name of the test material, the study code, used dose, the start and planned end

dates of the experiment, sex, and animal numbers. The rats were given standard granulated GLM fodder and tap water ad libitum.

The day before the start of the experiment, about 18–19 h before administration of the test material, the animals were left with no food, but water was still available. The food was given again 3 h following administration of the material.

In the preliminary experiment, one female was given the tested material in the form of an aqueous solution at a dose of 2000 $mg\,kg^{-1}$ b.w. and then to another female at a dose of 300 $mg\,kg^{-1}$ b.w. The material was administered as a single dose by using a metal intragastric catheter. A total of 0.5 mL of solution was given per 100 g of rat body mass.

In the main experiment following the preliminary study, the materials were administered to five female rats (including the one from the preliminary experiment) at a dose of 300 $mg\,kg^{-1}$ b.w. The preparation for administration, the administration procedure, and the administered volume corresponded to those used in the preliminary experiment.

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